

What is Claimed:

1. A mixture or set of sub-mixtures comprising X-mer precursors,
wherein the X-mer precursors have a minimum length of 3 nucleotides;
5 wherein the mixture has a minimum mixture coverage complexity of at least $56/N$ or wherein the set of sub-mixtures has a composite mixture coverage complexity of at least $56/N$, wherein N represents the number of distinct X-mer precursors in the mixture;
wherein each sub-mixture in said set has a reduced mixture coverage
10 complexity as compared with the composite mixture coverage complexity;
wherein each sub-mixture comprises a plurality of X-mer precursors;
wherein said length is selected independently for each X-mer precursor;
and
wherein the mixture or set of sub-mixtures further comprises a set of tags
15 wherein each tag is covalently linked to at least one X-mer precursor through a cleavable linker.
2. A mixture or set of sub-mixtures comprising X-mer precursors,
wherein said X-mer precursors have a minimum length of 3 nucleotides;
20 wherein said mixture has a minimum mixture coverage complexity of at least $56/N$ or wherein said set of sub-mixtures has a composite mixture coverage complexity of at least $56/N$, wherein N represents the number of distinct X-mer precursors in the mixture;
wherein each sub-mixture in said set has a reduced mixture coverage
25 complexity as compared with the composite mixture coverage complexity;
wherein each sub-mixture further comprises a plurality of X-mer precursors;
wherein said length is selected independently for each X-mer precursor;
wherein the mixture or set of sub-mixtures further comprises a set of tags
30 wherein each tag is covalently linked to at least one X-mer precursor through a cleavable linker; and
wherein said X-mer precursors have a determined isotopic composition.

3. The mixture or set of sub-mixtures of claim 1 or 2 wherein said mixture has a mixture coverage complexity of at least about 1/2 when said mixture contains at least 128 discrete X-mers, or wherein said set of sub-mixtures has a composite mixture coverage complexity of at least about 1/2 when said set of sub-mixtures contains at least 128 discrete X-mers.
4. The mixture or set of sub-mixtures of claim 1 or 2, wherein said mixture has a mixture coverage complexity of at least about 1/4 when said mixture contains at least 256 discrete X-mers, or wherein said set of sub-mixtures has a composite mixture coverage complexity of at least about 1/4 when said set of sub-mixtures contains at least 256 discrete X-mers.
5. The mixture or set of sub-mixtures of claim 1 or 2, wherein said mixture has a mixture coverage complexity of at least about 1/8 when said mixture contains at least 512 discrete X-mers, or wherein said set of sub-mixtures has a composite mixture coverage complexity of at least about 1/8 when said set of sub-mixtures contains at least 512 discrete X-mers.
6. The mixture or set of sub-mixtures of claim 1 or 2, wherein nucleotide sequences of the precursors of said mixture or set of sub-mixtures are known.
7. The mixture or set of sub-mixtures of claim 1 or 2, wherein a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is between approximately 10-100,000.
8. The mixture or set of sub-mixtures of claim 1 or 2, wherein a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is between approximately 20-20,000.

9. The mixture or set of sub-mixtures of claim 1 or 2, wherein a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is between approximately 20-10,000.
- 5 10. The mixture or set of sub-mixtures of claim 1 or 2, wherein a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is between approximately 20-5,000.
- 10 11. The mixture or set of sub-mixtures of claim 1 or 2, wherein a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is between approximately 50-1000.
- 15 12. The mixture or set of sub-mixtures of claim 1 or 2, wherein a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is greater than a mass number complexity (MNC) of a natural equivalent of the mixture or set of sub-mixtures, wherein the natural equivalent of the X-mer precursors are extended by one nucleotide, and wherein the number of tags in the set of tags is less than or equal to a number of X-mer precursors in the mixture or set of sub-mixtures.
- 20 13. The mixture or set of sub-mixtures of claim 1 or 2, wherein a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is greater than 75% of a mass number complexity (MNC) of a natural equivalent of mixture or set of sub-mixtures, wherein the natural equivalent of the X-mer precursors are extended by one nucleotide, and wherein the number of tags in the set of tags is less than or equal to a number of X-mer precursors in the mixture or set of sub-mixtures.
- 25 14. The mixture or set of sub-mixtures of claim 1 or 2, wherein a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is at least 0.5% of a number of X-mer precursors in the mixture or set
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of sub-mixtures, and less than or equal to the number of X-mer precursors in the mixture or set of sub-mixtures.

15. The mixture or set of sub-mixtures of claim 1 or 2, wherein a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is at least 1% of a number of X-mer precursors in the mixture or set of sub-mixtures, and less than or equal to the number of X-mer precursors in the mixture or set of sub-mixtures.

16. The mixture or set of sub-mixtures of claim 1 or 2, wherein a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is at least 10% of a number of X-mer precursors in the mixture or set of sub-mixtures, and less than or equal to the number of X-mer precursors in the mixture or set of sub-mixtures.

17. The mixture or set of sub-mixtures of claim 1 or 2, wherein a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is at least 25% of a number of X-mer precursors in the mixture or set of sub-mixtures, and less than or equal to the number of X-mer precursors in the mixture or set of sub-mixtures.

18. A method of analyzing a target nucleic acid sequence, comprising the steps of:
(1) hybridizing a mixture or set of sub-mixtures comprising tagged X-mer precursors to a target nucleic acid sequence,
wherein said mixture has a minimum mixture coverage complexity of at least $56/N$ or wherein said set of sub-mixtures has a composite mixture coverage complexity of at least $56/N$, wherein N represents the number of distinct X-mer precursors in the mixture,
wherein each sub-mixture in said set has a reduced mixture coverage complexity as compared with the composite mixture coverage complexity and further comprises a plurality of X-mer precursors,

wherein said length is selected independently for each X-mer precursor,

wherein the mixture or set of sub-mixtures further comprises a set of tags wherein each tag is covalently linked to at least one X-mer precursor through a cleavable linker, and

wherein said X-mer precursors comprise a 3'-end and a 5'-end,

- (2) processing said hybrids to alter the mass of said X-mer precursor portions of said hybrids in a target sequence-mediated reaction;
- (3) separating X-mer precursors with altered mass from X-mer precursors with unaltered mass;
- (4) cleaving said linkers to release the tags;
- (5) analyzing the released tags of step (4) via mass spectrometry; and
- (6) analyzing sequence of said target nucleic acid.

19. The method of claim 18 wherein the tags have a determined isotopic composition.

20. The method of claim 18, wherein in the step (1) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is between approximately 10-100,000.

21. The method of claim 18, wherein in the step (1) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is between approximately 20-20,000.

22. The method of claim 18, wherein in the step (1) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is between approximately 20-5,000.

23. The method of claim 18, wherein in the step (1) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is between approximately 50-1,000.

24. The method of claim 18, wherein in the step (1) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is greater than a mass number complexity (MNC) of a natural equivalent of the mixture or set of sub-mixtures, wherein the natural equivalent of X-mer precursors are extended by one nucleotide, and wherein the number of tags in the set is less than or equal to a number of X-mer precursors in the mixture or set of sub-mixtures.
25. The method of claim 18, wherein in the step (1) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is greater than 75% of a mass number complexity (MNC) of a natural equivalent of the mixture or set of sub-mixtures, wherein the natural equivalent of the X-mer precursors are extended by one nucleotide, and wherein the number of tags in the set is less than or equal to a number of X-mer precursors in the mixture or set of sub-mixtures.
26. The method of claim 18, wherein in the step (1) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is at least 0.5% of a number of X-mer precursors in the mixture or set of sub-mixtures; and less than or equal to the number of X-mer precursors in the mixture or set of sub-mixtures.
27. The method of claim 18, wherein in the step (1) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is at least 1% of a number of X-mer precursors in the mixture or set of sub-mixtures; and less than or equal to the number of X-mer precursors in the mixture or set of sub-mixtures.
28. The method of claim 18, wherein in the step (1) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is at least 10% of a number of X-mer precursors in the mixture or

set of sub-mixtures; and less than or equal to the number of X-mer precursors in the mixture or set of sub-mixtures.

29. The method of claim 18, wherein in the step (1) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is at least 25% of a number of X-mer precursors in the mixture or set of sub-mixtures; and less than or equal to the number of X-mer precursors in the mixture or set of sub-mixtures.

30. The method of claim 18, wherein in the step of hybridizing, said mixture has a mixture coverage complexity of at least about 1/2 when said mixture contains at least 128 discrete X-mers, and wherein said set of sub-mixtures has a composite mixture coverage complexity of at least about 1/2 when said set of sub-mixtures contains at least 128 discrete X-mers.

31. The method of claim 18, wherein in the step of hybridizing, said mixture has a mixture coverage complexity of at least about 1/4 when said mixture contains at least 256 discrete X-mers, or wherein said set of sub-mixtures has a composite mixture coverage complexity of at least about 1/4 when said set of sub-mixtures contains at least 256 discrete X-mers.

32. The method of claim 18, wherein in the step of hybridizing, said mixture has a mixture coverage complexity of at least about 1/8 when said mixture contains at least 512 discrete X-mers, or wherein said set of sub-mixtures has a composite mixture coverage complexity of at least about 1/8 when said set of sub-mixtures contains at least 512 discrete X-mers.

33. The method of claim 18, wherein in the step of hybridizing, said mixture is provided in at least two reaction mixtures.

34. The method of claim 18 further comprising the step of:

purifying the released tags of step (4) prior to analysis via mass spectrometry.

35. The method of claim 18 further comprising the step of:
5 separating the released tags of step (4) prior to analysis via mass spectrometry.
36. The method of claim 18 wherein steps (1) - (2) are conducted in solution.
- 10 37. The method of claim 18 wherein steps (1) - (2) are conducted with a surface-bound mixture.
38. The method of claim 18 wherein said released tags are analyzed via MS-MS mass spectrometry.
- 15 39. The method of claim 18 wherein said processing step comprises a target sequence mediated enzymatic assay.
40. The method of claim 39, wherein said enzymatic assay is an assay selected from a
20 polymerase extension assay and a ligase assay.
41. The method of claim 18, wherein said processing step comprises extending said hybridized X-mer precursors by polymerizing at least one nucleotide at said
25 3'-end of said hybridized X-mer precursors.
42. The method of claim 18, wherein said processing step comprises extending said hybridized X-mer precursors by polymerizing a single nucleotide at said 3'-end of said hybridized X-mer precursors.
- 30 43. The method of claim 42, wherein hybridized X-mer precursors are extended using an enzyme having a nucleotide polymerase activity.

44. The method of claim 42, wherein said nucleotide is a chain-terminating nucleotide triphosphate.

45. The method of claim 44, wherein said chain-terminating nucleotide triphosphate is a nucleotide selected from the group consisting of natural dideoxynucleotide triphosphates and mass-modified dideoxynucleotide triphosphates.

46. The method of claim 45, wherein the mass of said mass-modified dideoxynucleotide triphosphate is greater than that defined by the mass difference between the lightest and heaviest X-mer in the mixture.

47. The method of claim 18 wherein said processing step comprises ligating adjacent X-mer precursors using a DNA ligase.

48. The method of claim 18 wherein said processing step comprises ligating adjacent X-mer precursors using a condensing agent.

49. The method of claim 48, wherein said condensing agent is selected from the group consisting of carbodiimides and cyanogen bromide derivatives.

50. The method of claim 18 wherein said processing step comprises a chemical assay.

51. A method of analyzing a target nucleic acid sequence comprising steps of:
(1) hybridizing a target nucleic acid to a multiplicity of nucleic acid probes in an array comprising:

- a) a surface; and
- b) a multiplicity of nucleic acid probes, wherein the probes have 3'-OH ends, wherein the probes are attached to the surface at the 5' ends;

(2) hybridizing a mixture or set of sub-mixtures comprising tagged X-mer precursors to a target nucleic acid sequence,

wherein said mixture has a minimum mixture coverage complexity of at least $56/N$ or wherein said set of sub-mixtures has a composite mixture coverage complexity of at least $56/N$, wherein N represents the number of distinct X-mer precursors in the mixture,

wherein each sub-mixture in said set has a reduced mixture coverage complexity as compared with the composite mixture coverage complexity and further comprises a plurality of X-mer precursors,

wherein said length is selected independently for each X-mer precursor,

wherein the mixture or set of sub-mixtures comprises a set of tags wherein each tag is covalently linked to at least one X-mer precursor through a cleavable linker, and

wherein said X-mer precursors comprise a 3'-end and a 5'-end,

- (3) ligating said hybridized X-mer precursors located adjacent to said terminal 3' hydroxyl groups of said surface-bound probe to form a hybridized precursor/probe complex with said target nucleic acid sequence attached thereto; and
- (4) removing unligated X-mer precursors;
- (5) cleaving linkers to release said tags from said X-mer precursor said complex at said cleavable linker; and
- (6) analyzing said released tags via mass spectrometry to provide data on the sequence of the target nucleic acid.

52. The method of claim 51, wherein said mixture has a mixture coverage complexity of at least about $1/2$ when said mixture contains at least 128 discrete X-mers, and wherein said set of sub-mixtures has a composite mixture coverage complexity of at least about $1/2$ when said set of sub-mixtures contains at least 128 discrete X-mers.

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53. The method of claim 51, wherein said mixture has a mixture coverage complexity of at least about 1/4 when said mixture contains at least 256 discrete X-mers, or wherein said set of sub-mixtures has a composite mixture coverage complexity of at least about 1/4 when said set of sub-mixtures contains at least 256 discrete X-mers.
54. The method of claim 51, wherein said mixture has a mixture coverage complexity of at least about 1/8 when said mixture contains at least 512 discrete X-mers, or wherein said set of sub-mixtures has a composite mixture coverage complexity of at least about 1/8 when said set of sub-mixtures contains at least 512 discrete X-mers.
55. The method of claim 51, wherein nucleotide sequences of the X-mer precursors of said mixture or said set of sub-mixtures are known.
56. The method of claim 51, wherein said mixture is provided in at least two reaction mixtures.
57. The method of claim 51, wherein at least some of said mass-modified X-mer precursors comprise at least one mass tag or at least one chemical modification of a internucleoside linkage, a sugar backbone, or a nucleoside base.
58. The method of claim 51, wherein said hybridized X-mer precursor ligated with said probe using a DNA ligase.
59. The method of claim 51, wherein said hybridized X-mer precursor ligated with said probe using a condensing agent.
60. The method of claim 59, wherein condensing agent is selected from the group consisting of carbodiimides and cyanogen bromide derivatives.

61. The method of claim 51, wherein in the step of (2) hybridizing, the mixture comprises a set of tags, wherein the number of tags distinguishable by MS is between approximately 10-100,000.
- 5 62. The method of claim 51, wherein in the step of (2) hybridizing, the mixture comprises a set of tags, wherein the number of tags distinguishable by MS is between approximately 20-20,000.
- 10 63. The method of claim 51, wherein in the step of (2) hybridizing, the mixture comprises a set of tags, wherein the number of tags distinguishable by MS is between approximately 20-5,000.
- 15 64. The method of claim 51, wherein in the step of (2) hybridizing, the mixture comprises a set of tags, wherein the number of tags distinguishable by MS is between approximately 50-1,000.
- 20 65. The method of claim 51, wherein in the step (2) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is greater than a mass number complexity (MNC) of a natural equivalent of the mixture or set of sub-mixtures, wherein the natural equivalents of the X-mer precursors are extended by one nucleotide, and wherein the number of tags in the set is less than or equal to a number of X-mer precursors in the mixture or set of sub-mixtures.
- 25 66. The method of claim 51, wherein in the step (2) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is greater than 75% of a mass number complexity (MNC) of a natural equivalent of the mixture or set of sub-mixtures, wherein the natural equivalents of the X-mer precursors are extended by one nucleotide, and wherein the number of tags in the set is less than or equal to a number of X-mer precursors in the mixture or set of sub-mixtures.
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67. The method of claim 51, wherein in the step (2) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is at least 0.5% of a number of X-mer precursors in the mixture or set of sub-mixtures; and less than or equal to the number of X-mer precursors in the mixture or set of sub-mixtures.
68. The method of claim 51, wherein in the step (2) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is at least 1% of a number of X-mer precursors in the mixture or set of sub-mixtures; and less than or equal to the number of X-mer precursors in the mixture or set of sub-mixtures.
69. The method of claim 51, wherein in the step (2) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is at least 10% of a number of X-mer precursors in the mixture or set of sub-mixtures; and less than or equal to the number of X-mer precursors in the mixture or set of sub-mixtures.
70. The method of claim 51, wherein in the step (2) of hybridizing, a number of tags in the set of tags distinguishable by mass spectrometry after cleavage of the linkers is at least 25% of a number of X-mer precursors in the mixture or set of sub-mixtures; and less than or equal to the number of X-mer precursors in the mixture or set of sub-mixtures.
71. The method of claim 18 or 51, wherein said cleavable linker is a photocleavable linker.
72. The method of claim 18 or 51, wherein said cleavable linker is a chemical cleavable linker.
73. The method of claim 18 or 51, wherein said complexes are analyzed via MS-MS mass spectrometry.

- a. the mixture or the set of sub-mixtures of claim 1; and
- b. an enzyme having a nucleotide polymerase activity.

75. The kit of claim 74, further comprising a multiplicity of nucleotides selected from the group consisting of natural chain-terminating triphosphates and modified chain-terminating triphosphates.

76. The kit of claim 74, further comprising chain-terminating nucleotides with an affinity label for purification of nucleic acids.

77. A kit for carrying out a method of analyzing a target nucleic acid sequence comprising:

- a. the mixture or the set of sub-mixtures of claim 1; and
- b. a DNA ligase.

78. A kit for carrying a method of analyzing a target nucleic acid sequence, comprising:

- a. the mixture or the set of sub-mixtures of claim 1; and
- b. a condensing agent.

79. A kit for carrying out a method of analyzing a target nucleic acid sequence having a 3'-end and a 5'-end, comprising:

- a. the mixture or the set of sub-mixtures of claim 1;
- b. a DNA ligase; and
- c. an array comprising:
 - (a) a surface; and
 - (b) a multiplicity of nucleic acid sequence probes comprising:

- (i) a nucleic acid attached to said surface, wherein the nucleic acid has a terminal 3'-hydroxyl end and wherein the 5' end is directly or indirectly attached to said surface.

5 80. A kit for carrying out a method of analyzing a target nucleic acid sequence having a 3'-end and a 5'-end, comprising:

- a. the mixture or the set of sub-mixtures of claim 1;
- b. a condensing agent; and
- c. an array comprising:

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(a) a surface; and

(b) a multiplicity of nucleic acid sequence probes comprising:

- (i) a nucleic acid attached to said surface, wherein the nucleic acid has a terminal 3'-hydroxyl end and wherein the 5' end is directly or indirectly attached to said surface.

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